Detection of plasmid-mediated colistin resistance (mcr-1) in E. coli isolated from pig caecum in Austria

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Evaluation of tetracycline resistance genes during avian manure composting process

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Purpose: Antimicrobial resistances (AMRs) are an emerging threat for animal and public health. Among the different mechanisms of AMRs, plasmid-mediated antimicrobial resistance genes (PM-ARGs) are of major concern due to their capability of mobilization and transfer between bacteria. Since at least an 80% of bacteria are not cultivable, PM-ARGs should be studied directly in the microbiome, without previous culture.

AMRs are mainly studied in every step "from the farm to fork", but less known is how can they be spread into the environment through animal waste. Animal manure is commonly used as agricultural fertilizer and may carry on PM-ARGs which could reincorporate to food chain by crops. An interesting alternative is composting the animal manure, which reduces the presence of pathogenic bacteria and improves its quality as fertilizer, offering an added value to the product. In this work, we tested plasmid-mediated tetracycline resistance genes (tet) during four different small-scale avian manure composting processes.

Methods & Materials: Four different avian manure composting processes (avian manure+straw; avian manure+straw+fresh eggs; avian manure+straw+egg ashes; avian manure+straw+fresh eggs+egg ashes) at small scale were evaluated during eight weeks. Seven tet genes were quantified by real time PCR (*tet*(A), *tet*(B), *tet*(C), *tet*(K), *tet*(M), *tet*(Q) and *tet*(S)). Besides, the 16SARN gene was amplified for sample validation and to calculate the relative concentration of every gene (expressed as log of percentage of bacteria which carries a determinate gene). All genes, with the exception of *tet*(C), were detected.

Results: No apparent differences were observed among the types of composting. For every gene and every type of compost, there is an increase of gene quantity during the first week of composting. Also, there is a dramatic decrease of gene quantity during weeks 5-7, depending the gene. After this decrease, tet(M) and tet(S) genes were still under detection limit up to the final week; however, the rest of genes increase during the last weeks, to at least to initial levels.

Conclusion: Composting during 8 weeks doesn't seem to reduce the quantity of tet genes. Increase the number of weeks of composting as well as analyze other PM-ARGs will be evaluated. This work is funded by RTA2014-00012- C03-02.

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Detection of plasmid-mediated colistin resistance (*mcr-1)* in *E. coli* isolated from pig caecum in Austria

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Purpose: Colistin is regarded as a last line defence against infections caused by multidrug-resistant Gram-negative bacteria. Plasmid-encoded colistin resistance mediated by the mobile colistin resistance-1 (MCR-1) protein has been identified in food animal, human, food, and environmental isolates on every continent. We report on the first colistin-resistant *E. coli* strain documented in Austria.

Methods & Materials: A total of 257 pig caeca were collected at slaughterhouses in the framework of an EU monitoring program targeting antimicrobial resistance between January and December 2015 in Austria. Samples were screened for the presence of ESBL/AmpC producing *E. coli* using non-selective enrichment and McConkey agar supplemented with 1 mg/L cefotaxime. Antimicrobial susceptibility was determined by Sensititre microbroth dilution using EUVSEC and EUVSEC2 plates. The colistin-resistant isolate was sequenced using the Illumina MiSeq platform. Raw reads were *de novo* assembled based on Velvet algorithms using the pipeline available from the Center for Genomic Epidemiology (CGE). The assembled genome was analyzed using pipelines MLST, PlasmidFinder, pMLST, and ResFinder available from CGE.

Results: In November 2015, an *E. coli* isolate harboring the *mcr-1* gene was isolated from pig caecum sampled in a slaughterhouse in Austria. The isolate belonged to MLST sequence type 101 (ST101) and showed a MIC value of 8 mg/L for colistin. Besides *mcr-1*, the isolate contained seven additional resistance genes ($bla_{CTX\text{-}M\text{-}1}$, *aadA5*, *dfrA17*, *strA, strB*, *sul*2, and *tet*(B)) conferring resistance to extended-spectrum beta-lactam antibiotics as well as aminoglycosides, tetracyclines, trimethoprim, and sulfamethoxazole. Plasmid replicons detected were IncFIB(AP001918), IncI1, IncFIC(FII), p0111, IncX4, and ColRNAI. The Inc groups identified were IncI1[ST-3] and IncF[F46:A-:B24]. Analysis of contigs showed that *mcr-1*, insertion sequence IS*Apl*1, and replicon IncX4 were located on the same contig indicating that *mcr-1* might be situated on plasmid IncX4.

Conclusion: The detection of the *mcr-1* gene in an *E. coli* strain isolated from a pig shows its presence in livestock in Austria. In Austria, the implementation of a selective screening program in existing monitoring programs will be necessary to ascertain the true prevalence of plasmid-mediated colistin resistance in *Enterobacteriaceae* in livestock and food.

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